BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON VISCERAL GOUT IN LAYERS WITH TRIAL OF TREATMENT

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ABSTRACT

A commercial 35 week old layer flock located in Salhia city Sharkia governorate suffered from drop in egg production, increased water consumption, diarrhea and increased mortality rate after consuming feed misformulation contain over 5kg sodium bicarbonate (SB) instead of 1-2 kg per/ton feed. Post mortem examination revealed deposition of whitish chalky material in the kidneys and coating of serous surface of abdominal organs especially liver and heart by this material. A total of one hundred layer hens were divided into four equal groups. Group 1 was obtained from healthy flock, hens received normal commercial ration and served as a control group. Hens of group (2,3 and 4) were obtained from affected flock. Group 2 received misformulated ration, group 3 received corrected ration free from sodium bicarbonate and group 4 received corrected ration with addition of allopurinol at a dose level 40 mg/Kg. b. wt and the samples were taken at the beginning of the experiment and at two and three weeks later. The hematological parameters RBCs count, Hb concentration and PCV of affected hens were significantly increased compared with healthy ones, moreover, blood pH in affected hens shifted toward the alkaline side. Serum levels of uric acid, total proteins and sodium were significantly increased while potassium level and chloride were relatively decreased. Microscopic examination revealed severe renal changes in highly affected hens, these changes included coagulative necrosis and renal casts in the lumen of renal tubules. The heart showed zenkers degeneration of cardiac muscle fibers and edema.
Correction of ration improves partially the measured parameters whereas addition of allopurinol with correction of ration normalizes the examined parameters. It was concluded that correction of ration with addition of allopurinol, leads to normalization of serum uric acid and others examined parameters in laying hen.

INTRODUCTION

The kidney in bird can be affected by a number of specific diseases and disorders. One of the important disorders associated with kidney damage is gout. Avian gout is a metabolic condition in which kidney function has decreased to the point where uric acid or urate accumulates in soft tissues of various organs in the body. There are two forms of avian gout (visceral and articular). Visceral gout present if the urates are deposited in the viscera and articular gout if the urates are deposited around the joint (Saif et al., 2003).

Visceral gout cause production loses and death in turkey (Tang et al; 2005), laying and broiler chickens (Rahman and Samad 2004), geese (Palya et al; 2004), guinea fowl (Cooper et al; 1996) and quail (Das et al; 1992).

In birds, uric acid is the end product of nitrogen metabolism. It is converted in mammals to less harmful substances with the help of enzyme uricase. This enzyme is absent in birds, Hence uric acid is the final excretory product (Osbaldiston 1968). Uric acid is produced mainly in liver and excreted by tubular excretion of the kidney (Harison and Harison 1986). High blood level of uric acid favor its precipitation in kidneys leading to tubular damage that cause hyperuricemia which is characterized by obstruction of the ureter and its branches (Mubarak and Sharkawy 1999).
In gout, blood levels of uric acid can reach 44 mg/100ml as compared to 5-7 mg/100 ml in normal birds (Harison and Harison 1986). Outbreak of visceral gout in poultry occurs due to multietiological factories; dietary causes as excessive uses of sodium in water and feed (Mubarak, 2004); excess of protein more than 30-40% in feed (Tang et al; 2005); excess dietary calcium more than 3% with low available phosphorous less than 0.4% (Mendez et al 2001) and prolonged vitamin A deficiency (Siller, 1981) another causes may be mycotoxins such as oosporein (Pegram and Wyatt 1981). Also infectious disease as infectious bronchitis (Kang et al; 1999).

There is a little literature about treatment of visceral gout in birds. Dietary acidification with potassium chloride or methionine may be benefit for decreasing kidney lesions (Saif et al; 2003). Use of electrolytes or diuretics through water may assist in controlling mortality caused by gout (Upendra 1991). Medicinal plants play an important role for controlling mortality caused by gout (Chatterjee and Misra 2004). Also drugs, which lower plasma uric acid levels, can be used for treatment of visceral gout; these drugs can be classified according to their mode of action as uric acid inhibitors (Allopurinol and Tisopurine); uric acid execrators (Probenecid, Benzbromarone) and uric acid lyser (Urate- Oxidase) (Pawlotsky 1994).

In the present study, biochemical and histopathological changes in laying hens suffering from visceral gout caused by salt poisoning were investigated. And trial for correction of side effects of gout by allopurinol was done.

**MATERIALS AND METHODS**

Two layers flocks in Salhia city, Sharkia province, each contain six thousand birds of 35 week age with average body weight 1.75 kg were used.
During routine observation of the farms, there is drop in egg production, increased water consumption, diarrhea and increased daily mortality (15 bird/day) in one farm of them. Post mortem examination showed that all dead birds were affected by visceral gout. From a case history about composition of ration, it is clear that the flock was fed on ration erroneously formulated to contain sodium bicarbonate (SB) over 5Kg/Ton instead of 1-2 kg/ton. This ration called (Ration II). Ten highly affected hens were sacrificed to confirm lesions specific for visceral gout. Another farm was fed on normal commercial layer ration contain 1 kg SB /ton (Ration I). The healthy condition, mortality rate and its production was normal.

**Drug:** Allopurinol (allopurinol 300) produced by EIPICO; Egyptian Int. pharmaceutical industries co. 10th of Ramadan city A.R.E.

It was added to feed at a dose level 40 mg /kg b.wt according to *Melvin etal; (2002).*

**Rations:** There are four types of ration were used in our experiment.

**Ration I:** is normal commercial layer ration containing one kg of SB /ton.

**Ration II:** is layer ration containing 5 kg SB/ton (misformulated ration).

**Ration III:** is commercial layer ration free from SB (corrected ration).

**Ration IV:** is commercial layer ration free from SB and contain allopurinol at a dose level 40 mg/kg. b. wt.

**1-Experimental design:-**

One hundred laying hens were used for this study, 25 of them were obtained from healthy flock and 75 from affected flock with visceral gout. All birds were divided into four equal groups.
1\textsuperscript{st} group: served as healthy control, fed on ration I contain 1Kg SB/ton.

2\textsuperscript{nd} group: served as affected hens fed on ration II contain 5 Kg SB/Ton.

3\textsuperscript{rd} group: served as affected hens fed on ration III free from SB.

4\textsuperscript{th} group: served as affected hens fed on ration IV free from SB and contain allopurinol.

Each bird received about 120 gm feed/day. Water ad-libitum was supplied to all groups. The experimental period continue for 3 weeks.

2- Blood samples:

Were obtained from wing vein from 5 birds of each group at three different durations. 1\textsuperscript{st} at the beginning of the experiment 2\textsuperscript{nd} and 3\textsuperscript{rd} after two and three weeks from the beginning of the experiment respectively.

3- Hematological and biochemical studies:

Two blood samples were collected from each bird of each group. The first sample was collected on sodium salt of EDTA for hematological studies, RBCs counts Hb concentration and PCV were estimated according to the method described \textit{Jain (1986)}, blood PH was determined using \textit{Whatman indicator papers}. The second sample was used for serum separation, serum samples were used for determination of serum uric acid \textit{(Trinder; 1969)}. Serum total protein \textit{(Doumas and Biggs 1972)}, serum sodium and potassium measured by flam photometer \textit{(Bauer; 1982)}, serum chloride \textit{(Felid Kamb, 1974)}.

4- Histopathological examinations:

Tissue specimens from kidneys and heart of sacrificed bird were fixed in10\% formalin, processed and stained with (H&E) for microscopic examinations \textit{(Bancroft and Stevens, 1982)}.

5- Statistical analysis:

The data obtained were statistically analyzed \textit{(Tamhane and Dunlop, 2000)}.
RESULTS AND DISCUSSION

Visceral gout is a metabolic disorder that results from hyperuricemia. It is characterized by precipitation of urates in kidney and on serous surfaces of abdominal organs causing loss of production and high mortality in laying hens (Riddell 1997).

Causes of visceral gout may be nutritional as excessive use of sodium bicarbonate which is added to feed to improve egg shell quality (Howes 1966 and charles et al 1972) and decreases losses due to heat stress (Branton et al 1986). The present results indicated that laying flock fed on ration misformulation contain high level of SB (over 5kg/ton) results in typical lesions of visceral gout. Highly affected hens were emaciated and severely congested with subcutaneous edema, they showed white chalk-like deposits covering the surface of various abdominal organs as well as the heart sac (Fig. 1). Kidneys were pale with irregular and excessive enlargement of lobules. Ureters were distended with urates (Fig. 2). These observations are mimic with those described by (Davison & Wideman 1992) and (Riddell1997) in commercial layers spontaneously intoxicated by sodium bicarbonate.

Concerning the hematological parameters (Table 1):- There was a significant (P≤ 0.01) increase in hemoglobin concentration, RBCs counts and packed cell volume of affected layer bird compared with the healthy ones. The significant increase in the hematological parameters may be due to diarrhea, ascitis and dehydration of the affected bird (Sander et al 1998) due to toxicity of sodium sesquicarbonate toxicity in broiler chickens.

Blood PH in the affected birds was shifted toward the alkaline side. This may be due to metabolic alkalosis created by bicarbonate overload in the affected birds.

Similar results were obtained by Bottje and Harrison (1985b), Mubarak and Sharkawy (1998) and Squires and julian (2001).

In this study correction the ration improves partially the hematological variance whereas use of allopurional with the corrected ration normalizes the examined hematological parameters.

Concerning the biochemical parameters (Table2): serum uric acid showed highly significant increase (P≤0.001) in the affected bird compared to the healthy ones.

Hyperuricaemia is related to breakdown, and turnover of nucleoprotein resulting in overproduction of acid, which is accelerated in the presence of renal damage, that results in diminished excretion by renal tubules (Brown, 1996) both mechanisms will result in Hyperuricaemia.

Hyperuricaemia is an obvious manifestation of gout (Baur and krane, 1964). Similar result was obtained by Mubarak and sharkawy (1998), and Sahar and kawkab (2004) in laying pullets and broiler due to sodium bicarbonate toxicity.

This study revealed highly significant increase (P≤0.001) in sodium level, whereas potassium and chloride were significantly decreased (P≤0.01) in the affected bird compared with the healthy control (Tab 2).

The increased sodium levels in the affected layers may be due to high sodium intake (Tietz et al 1998) and inability of the birds to excrete it (Riddell, 1997).
In this study the hypernatraemia was associated by hypokalemia. This may be due to strong relation between sodium and potassium as \( k^+ \) is essential for extrusion of \( Na^+ \) from tissue cell (Cogan, 1991; and Tohda, 1994). In this study the decreased serum levels of \( K^+ \) and \( cl \) may be due to increased excretion of these elements due to renal dysfunction and loss of these elements in the watery dropping of the affected bird (Mubarak and Sharkawy, 1998).

Similar changes of blood parameters including metabolic alkalosis, hypernatremia, hypokalemia and hypochloremia were reported in commercial layers exposed to sodium bicarbonate (SB) toxicity (Davison & Wideman, 1992). Also other animals and even human beings exposed to SB toxicity showed similar changes of blood chemistry (Thomas & Stone 1994; Rivas et al; 1997). This agree with that obtained by Sahar and Kawkab (2004) and EJaz et al (2005) in broilers.

The present study revealed highly significant increase \((P \leq 0.001)\) in total protein in the affected layers bird compared with the healthy control, this increase may be due to fluid losses by diarrhea and dehydration due to sodium bicarbonate toxicity.

Similar results were obtained by Mert (1991) in laying and broiler chickens' gout, and Sander et al (1998) due to sodium sesquicarbonate toxicity in broiler chickens.

The changes in the biochemical parameters were confirmed by histopathological changes in the kidney and heart of affected layers. The kidney of affected layers showed coagulative necrosis and renal casts in the lumen of renal tubules (Fig. 3). Other cases showed focal aggregation of mononuclear cells and few fibroblasts among necrotic renal tubules (Fig. 4).
The heart of affected layers showed hyaline degeneration, necrosis and destruction of muscle fibers, in addition to edema and round cells infiltration among the degenerated muscle fibers (Fig. 5). Other cases revealed zeners degeneration of cardiac muscle fibers and edema with few leuocytic cells infiltration (Fig.6).

Similar results were obtained by Mubarak and Sharkawy (1998), and Mubarak (2004) due to toxicity induced by sodium bicarbonate.

Correction of the ration improves all examined biochemical parameters. Normalization of serum uric acid and other examined biochemical parameters achieved by dietary regiment with additional allopurinol.

Allopurinol is the powerful inhibitor of xanthine oxidase enzyme responsible for the oxidation of xanthine and hypoxanthine to uric acid and urate formation. It reduces urates and uric acid concentration in both body fluids and urine and reduces risk of renal impairment (Pawlotsky 1994). Similar results were obtained by van. Doornum and Ryan (2000) who stated that allopurinol is the drug of choice for urate lowering therapy in the presence of renal impairment, also Klandrof et al (2001) and Melvin et al (2002) stated that allopurinol with a dose level 40 or 50 mg/kg. B.wt. for 21 days by fed rout can lower plasma uric acid. Akkasilpa et al (2004) reported that the allopurinol is superior in hyperuricemic patients.

This improvement is confirmed by the improve in the pathology of the kidneys and heart. The kidney of treated layers showed mild degenerative changes in renal tubules (Fig. 7). The heart of treated layers showed mild congestion of cardiac blood vessels (Fig.8).

It was concluded that correction of ration with addition of allopurinol. Leads to normalization of serum uric acid and others examined parameters in laying hen.
جدول بالعرض (1)
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جدول بالعرض (2)
(Fig. 1): affected layers showing white chalk-like deposits covering the surface of various abdominal organs as well as the heart sac.

(Fig. 2): Kidneys of affected layers showing pale irregular and excessive enlargement of lobules, ureters were distended with urates
(Fig. 3): The kidney of affected layers showing coagulative necrosis and renal casts in the lumen of renal tubules (H&E X 300)
(Fig. 4): The kidney of affected layers showing focal aggregation of mononuclear cells and few fibroblasts among necrotic renal tubules (H&E X 1200)

(Fig. 5): The heart of affected layers showing hyaline degeneration, zenkers necrosis and destruction of muscle fibers, edema and round cells infiltration among the degenerated muscle fibers (H&E X 1200)
(Fig. 6): The heart of affected layers showing Zenkers degeneration of cardiac muscle fibers and edema with few leucocytic cells infiltration (H&E X 300).

(Fig. 7): The kidney of treated layers showing mild degenerative changes in renal tubules (H&E X 120).
(Fig. 8): The heart of treated layers showing mild congestion of cardiac blood vessels (H&E X 120).
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**دراسات كيميائية وباثولوجية على نقرس الدجاج البياض ومحاولة علاجه**

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قسم الأدوية

عمل بحوث صحة الحيوان بالنزقازيق

تم إجراء هذا البحث على مزارع دجاج بياض عمرها 35 أسبوع بمدينة الصالحية-محافظة الشرقية، حيث وجد أحد المزارع به انخفاض ملحوظ بالإنتاج اليومي للبيض مع زيادة في استهلاك معدل مياه الشرب وإسهال مائي بالإضافة إلى ارتفاع معدل الناقفة اليومي. وبإجراء الدراسة التشريحيتين بين وجود ترسبات طباشيرية على الأعضاء الداخلية للدجاج المصاب وتضخم شديد بالحاليين والكلفي ورضاة أسباب هذه الحالة اتضح أن هناك خطأ في تركيبه العلف حيث تضاعف بيكترونات الصوديوم بمعدل أكثر من 5 ك/طن علف وقياس حامض البوليك في السيرم وجد أنه مرتفع ارتفاعاً ملحوظاً مما يوضح إصابة القطيع بالنقرس الحشوي.
وقد أجريت هذه التجربة على فئات دجاجة بياض عمر 35 أسبوع (25 دجاجة سليمة و 75 دجاجة مصابة) وقسمت إلى 4 مجاميع متساوية في أفقيان منفصلة على النحو التالي.

المجموعة الأولى: دجاج سليم يتغذى على علبة بياض تحتوي على كيلو بيكربونات الصوديوم/طن (ضابطة).

المجموعة الثانية: دجاج مصاب يتغذى على علبة بياض تحتوي على 5 كيلو بيكربونات الصوديوم/طن.

المجموعة الثالثة: دجاج مصاب يتغذى على علبة بياض خالية من بيكربونات الصوديوم.

المجموعة الرابعة: دجاج مصاب يتغذى على علبة بياض خالية من بيكربونات الصوديوم وتحتوي على ألبيورنول بمعدل 40 مجم/كم من وزن الطائر.

استمر العلاج والملاحظة لمدة 3 أسابيع وتم أخذ عينات دم للفحص الكيميائي والباثولوجي في بداية التجربة وبعد أسبوعين وثلاثة أسابيع واتضح من النتائج مايلي:

- زيادة معنوية في عدد كرات الدم الحمراء ونسبة هيموجلوبين الدم وحجم الخلايا الضغوفة بالدجاج المصاب مقارنة بالدجاج السليم كما أن درجة الحوضة PH اتجهت نحو القلوية. كما وجد خلل في وظائف الكلى حيث اقترن بارتفاع حامض البوليك والبروتين الكلى والصوديوم في الدم.

المصاب مقارنة بالدجاج السليم. وتتغير العلبة للدجاج المصاب بطريقة خالية من بيكربونات الصوديوم حدد تحسن جزئي في القياسات السابقة وعند إضافة عقار ألبيورنول مع تغير العلبة حدث تحسن.

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كامل في القياسات السابقة وخاصة انخفاض معنوي في حامض البوليك في الدجاج المعالج مقارنة بالدجاج المصاب.

بما سبق يتضح إمكانية استعمال عقار الوبيرونول بجرعة 40 مجم/ كجم وزن الطائر كعلاج للدجاج المصاب بالنقرس الناتج عن التسمم ببكتيريونات الصوديوم.